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ORIGINAL REPORT

Reducing Unnecessary Biopsy During Prostate Cancer Screening Using a Four-Kallikrein Panel: An Independent Replication

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See accompanying editorial on page 2491

A B S T R A C T

Purpose

We previously reported that a panel of four kallikrein forms in blood—total, free, and intact prostate-specific antigen (PSA) and kallikrein-related peptidase 2 (hK2)—can reduce unnecessary biopsy in previously unscreened men with elevated total PSA. We aimed to replicate our findings in a large, independent, representative, population-based cohort.

Patients and Methods

The study cohort included 2,914 previously unscreened men undergoing biopsy as a result of elevated PSA (≥ 3 ng/mL) in the European Randomized Study of Screening for Prostate Cancer, Rotterdam, with 807 prostate cancers (28%) detected. The cohort was randomly divided 1:3 into a training and validation set. Levels of kallikrein markers were compared with biopsy outcome.

Results

Addition of free PSA, intact PSA, and hK2 to a model containing total PSA and age improved the area under the curve from 0.64 to 0.76 and 0.70 to 0.78 for models without and with digital rectal examination results, respectively ($P < .001$ for both). Application of the panel to 1,000 men with elevated PSA would reduce the number of biopsies by 513 and miss 54 of 177 low-grade cancers and 12 of 100 high-grade cancers. Findings were robust to sensitivity analysis.

Conclusion

We have replicated our previously published finding that a panel of four kallikreins can predict the result of biopsy for prostate cancer in men with elevated PSA. Use of this panel would dramatically reduce biopsy rates. A small number of men with cancer would be advised against immediate biopsy, but these men would have predominately low-stage, low-grade disease.

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H.L. holds patents for free PSA and hK2 assays, and with K.P., is named as co-inventor on a patent application for intact/nicked PSA assays.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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INTRODUCTION

Prostate-specific antigen (PSA) testing is one of the most widely used screening tests for cancer. It has been estimated that 75% of US males older than age 50 years have had at least one PSA test, with 50% undergoing regular PSA screening.[1] The value of PSA testing in men who would otherwise not be screened was recently assessed in the European Randomized Study of Screening for Prostate Cancer (ERSPC). The authors reported that although PSA screening was significantly associated with a 20% reduction in cancer-specific mortality at a median of 9 years of follow-up, this benefit came at a relatively high cost, with large numbers of men needing to be screened, biopsied, and treated to save one life.[2] This is also apparent from US data. For example, using estimates of the number of prostate biopsies per year[3] and the annual incidence of prostate cancer,[4] we estimate that each year, PSA testing leads to approximately 750,000 unnecessary biopsies for prostate cancer in the United States.

One way to reduce the harm of PSA testing and thus shift the ratio between benefits and harms would be to improve its moderate predictive value[5] and thus reduce unnecessary biopsy. Using a data set from the Göteborg, Sweden section of the ERSPC, we recently reported that a panel of four kallikreins—free, intact, and total PSA and kallikrein-related peptidase 2 (hK2)—was markedly more accurate than PSA alone in predicting the outcome of prostate biopsy. We estimated that using the panel to determine referral to biopsy would reduce biopsy rates by 573 per 1,000 men with elevated

PSA, with only a small number of men with cancer (n = 42) being advised against immediate biopsy.[6]

The gold standard for any prostate cancer marker is independent replication in a large, population-based cohort of men representative of those to whom the marker would be applied in clinical practice, such as men with elevated PSA who are considering biopsy. Therefore, we report here a replication of these findings on an independent data set, the Rotterdam section of the ERSPC.

PATIENTS AND METHODS

Patient Cohort

The ERSPC involved several rounds of screening. The first round constituted a participant's first PSA test in the ERSPC, and rounds 2 and 3 were the second and third PSAs taken 4 and 8 years later. In total, 19,970 men age 55 to 75 years participated in the first screening round of ERSPC Rotterdam (94% participation) during 1993 to 2000. The screening protocol and preliminary features of the first screening round have been described elsewhere.[7] Because our scientific question relates to elevated PSA, we excluded 1,090 men biopsied for reasons other than an elevated PSA (abnormal findings on digital rectal examination [DRE] in 41%, on transrectal ultrasound [TRUS] in 41%, and on both in 18%). Of the 3,423 men with an elevated PSA, 3,028 (88%) were biopsied. We excluded 114 men (4%) whose kallikreins could not be measured as a result of insufficient frozen blood samples. Clinical stage was determined by using both TRUS and DRE results.

Laboratory Methods

Laboratory methods were the same as those detailed in our prior publication,[6] with an important modification of the protocols used to measure intact PSA and hK2. These protocols used $F(ab')_2$ fragments of the monoclonal capture antibodies to significantly reduce the frequency of nonspecific assay interference.[8] Serum samples were retrieved from the archival serum bank in Rotterdam (where they had been stored frozen at −80°C after their initial processing within 3 hours from venipuncture) and shipped frozen on dry ice to Malmö, Sweden in 2005 to 2007. Analyses of free, total, and intact PSA and hK2 were performed in H.L.'s laboratory at the Wallenberg Research Laboratories, Department of Laboratory Medicine, Lund University, Malmö University Hospital in Malmö, Sweden during 2005 and 2007. Free and total

Table 1. Clinical Characteristics of Training and Validation Sets

Characteristic	Training Set				Validation Set			
	No Cancer (n = 526)		Cancer (n = 202)		No Cancer (n = 1,581)		Cancer (n = 605)	
	No. of Participants	%	No. of Participants	%	No. of Participants	%	No. of Participants	%
Age at venipuncture, years								
Median	67		67		66		67	
Interquartile range	62-70		62-70		62-70		63-71	
Total PSA, ng/mL								
Median	4.80		6.53		4.77		6.13	
Interquartile range	3.91-6.42		4.42-11.4		3.84-6.43		4.34-10.4	
Free PSA, ng/mL								
Median	1.03		1.05		1.05		1.01	
Interquartile range	0.74-1.45		0.70-1.52		0.77-1.46		0.69-1.51	
Intact PSA, ng/mL								
Median	0.49		0.56		0.49		0.53	
Interquartile range	0.34-0.66		0.38-0.85		0.35-0.68		0.38-0.83	
Kallikrein-related peptidase 2, ng/mL								
Median	0.067		0.085		0.066		0.083	
Interquartile range	0.044-0.092		0.055-0.128		0.046-0.096		0.057-0.122	
DRE result								
Normal	432	82	111	55	1,282	81	324	54
Abnormal	94	18	91	45	299	19	281	46
TRUS result								
Normal	438	83	108	53	1,329	84	331	55
Abnormal	88	17	94	47	252	16	274	45
Clinical stage								
T1c			89	44			245	40
T2a			45	22			171	28
T2b			10	5			45	7
T2c			16	8			41	7
T3 or T4			42	21			103	17
Biopsy Gleason grade								
≤ 6			128	63			384	63
7			57	28			178	29
≥ 8			15	7			38	6
Not available*			2	1			5	1

Abbreviations: PSA, prostate-specific antigen; DRE, digital rectal examination; TRUS, transrectal ultrasound.
*Not available for seven participants diagnosed with cancer before Gleason grading was used (one participant in 1993 and six participants in 1994). Anderson grade was available for these participants. In the training set, two patients were grade 2. In the validation set, two patients were grade 1, and three patients were grade 2.

PSA were measured using the dual-label DELFIA Prostatus Total/Free PSA-Assay (Perkin Elmer, Turku, Finland), whereas the measurements of intact PSA and hK2 were performed as previously reported.[8] The intact PSA assay measures only free, uncomplexed intact PSA (ie, not cleaved at Lys_{145}-Lys_{146}). All analyses were conducted blind to biopsy result.

Statistical Methods

Our principal analysis was to determine whether additional kallikreins (free PSA, intact PSA, and hK2) could enhance discrimination of prostate cancer diagnosis in previously unscreened men with elevated PSA when compared with a base laboratory model (including age and total PSA) or a base clinical model (including age, total PSA, and DRE result). Each model gives a predicted probability of a positive biopsy. We evaluated the increment in predictive accuracy when all additional kallikreins were added to the base model to form a model that included four kallikreins. Predictive accuracy was given as the area under the receiver operating characteristic curve (AUC). High-grade cancer was defined as Gleason grade 7 or higher or Anderson grade 2 or higher when Gleason grade was not available. The AUC for high-grade cancer was calculated from the predicted probabilities of any cancer (ie, we did not build a separate model for the outcome of high-grade cancer); in this analysis, patients with Gleason grade 6 and lower tumors were classified the same as patients with negative biopsy. All markers were entered into all models using restricted cubic splines to model any possible nonlinear relationship with outcome.

We previously reported on the predictive accuracy of these kallikreins using data from men biopsied in the first screening round of ERSPC Göteborg, Sweden.[6] Our initial plan was to use the Rotterdam data to independently validate those models built using the Göteborg data. However, because of the changes in assay technique described earlier, we randomly split the Rotterdam data set into training and validation data sets. The training data set contained one fourth of the Rotterdam data (n = 728), and the validation data set contained the remaining three fourths (n = 2,186). The random allocation was performed by stratifying on cancer diagnosis (any and high-grade diagnosis); no other variables, such as biomarker levels, were used for stratification.

The models built with the training set were finalized on April 30, 2008 and sent to a third party on May 15, 2008; the validation set was then opened for the independent evaluation of model performance. We evaluated the performance of our models by calculating the AUC. CIs and inference statistics for differences between AUCs were obtained using the method of DeLong.[9] To characterize the clinical effects of the models, we used decision curve analysis.[10] This method estimates a net benefit for prediction models by summing the benefits (true positives) and subtracting the harms (false positives), where the latter is weighted by a factor related to the relative harm of a missed cancer compared with an unnecessary biopsy. A model is of clinical value if it has the highest net benefit across the full range of threshold probabilities at which a patient would choose to be biopsied. All numbers and figures given here are for the independent validation set. Statistical analyses were conducted using Stata 10.0 (StataCorp, College Station, TX).

RESULTS

In total, 807 (28%) of 2,914 eligible, unscreened participants with elevated total PSA in serum (≥ 3 ng/mL) were diagnosed with prostate cancer. The training set contained 728 participants; overall, 202 participants (28%) were diagnosed with prostate cancer, and 74 participants (10%) were diagnosed with high-grade disease (Table 1). The independent validation set contained 2,186 participants, with similar rates of total (28%, n = 605) and high-grade (10%, n = 219) prostate cancer. For men diagnosed with prostate cancer, biomarker measurements were similar between the validation and training sets, as were DRE and TRUS results. These characteristics were also comparable between the two sets for participants without a prostate cancer diagnosis.

The predictive accuracy of the two separate laboratory and clinical models built using the training set when independently evaluated using the validation set is shown in Table 2 and Appendix Figures A1 and A2 (online only). For prediction of any prostate cancer, the laboratory base model (PSA and age, although age was not found to be predictive) had an AUC of 0.637 (95% CI, 0.609 to 0.664), which increased to 0.764 (95% CI, 0.739 to 0.788) for the full laboratory model (age plus kallikrein panel). Corresponding data for the clinical models, which incorporate DRE, were 0.695 (95% CI, 0.668 to 0.721) for the base model (age, DRE, and PSA) and 0.776 (95% CI, 0.752 to 0.799) for the full model (age, DRE, and kallikrein panel). The enhancements of the full models to base models to predict any cancer were statistically significant (all $P < .001$). Applying these models to

Table 2. Predictive Accuracy of Models Built on the Training Set When Applied to the Independent Validation Set

Predictor	Any Cancer			High-Grade Cancer		
	AUC	95% CI	*P* (*v* base)	AUC	95% CI	*P* (*v* base)
Laboratory model						
Laboratory base model	0.637	0.609 to 0.664	—	0.776	0.741 to 0.812	—
Laboratory base + free PSA	0.727	0.701 to 0.752	< .001	0.832	0.801 to 0.863	< .001
Laboratory base + intact PSA	0.635	0.607 to 0.663	.9	0.774	0.736 to 0.812	.8
Laboratory base + nicked PSA	0.769	0.745 to 0.793	< .001	0.844	0.814 to 0.874	< .001
Laboratory base + hK2	0.648	0.621 to 0.675	.14	0.775	0.740 to 0.811	.9
Full laboratory model	0.764	0.739 to 0.788	< .001	0.825	0.791 to 0.860	.008
Clinical model						
Clinical base model	0.695	0.668 to 0.721	—	0.806	0.772 to 0.841	—
Clinical base + free PSA	0.752	0.727 to 0.776	< .001	0.851	0.822 to 0.879	< .001
Clinical base + intact PSA	0.694	0.668 to 0.721	.9	0.809	0.774 to 0.844	.7
Clinical base + nicked PSA	0.784	0.761 to 0.807	< .001	0.860	0.833 to 0.888	< .001
Clinical base + hK2	0.702	0.676 to 0.728	.16	0.808	0.773 to 0.842	.8
Full clinical model	0.776	0.752 to 0.799	< .001	0.837	0.803 to 0.870	.08

NOTE. The base model for the laboratory model includes age and total PSA, and the base model for the clinical model includes age, total PSA, and digital rectal examination result. The full model includes the base model plus free PSA, intact PSA, and hK2. Cancers with biopsy Gleason grade of ≥ 7 were considered high grade. Nicked PSA is calculated from free PSA minus intact PSA; it would be redundant to include both in the full model.

Abbreviations: AUC, area under the curve; PSA, prostate-specific antigen; hK2, kallikrein-related peptidase 2.

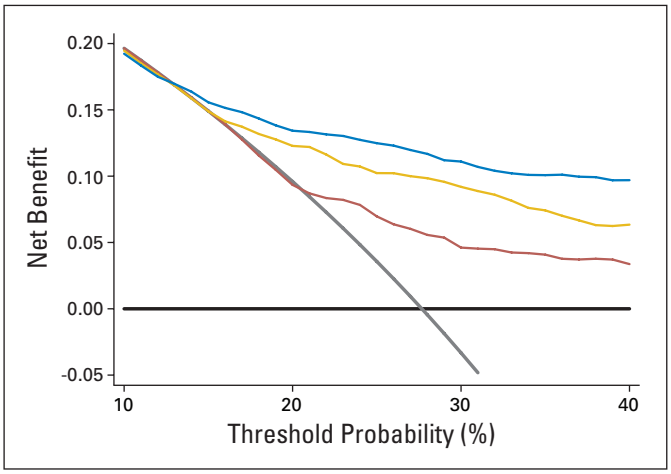

Fig 1. Decision curve analysis for the laboratory model. The red line is for base model (age and total prostate-specific antigen [PSA]); the gold line is for base model plus free PSA; and the blue line is for full model (age, total PSA, free PSA, intact PSA, and kallikrein-related peptidase 2). As comparison, the gray line represents the strategy of biopsying all men, and the black line represents the strategy of biopsying no men. The line with the highest net benefit at a particular threshold probability will lead to the best clinical results.

high-grade (Gleason grade $\geq$ 7 at biopsy) disease aided discrimination, although only the addition of free PSA aided the discrimination significantly ($P < .001$), whereas intact PSA and hK2 did not seem to add important predictive value.

To put these results in a clinical context, we plotted decision curves for any prostate cancer diagnosis (Figs 1 and 2). The net benefit of the full model was superior for both laboratory and clinical settings for all threshold probabilities greater than 13%. In other words, use of the model would be of benefit for men who would accept biopsy if their risk of cancer was approximately one in seven or higher but would refuse biopsy if their risk was lower than this threshold. Men who are very risk averse—who would choose biopsy even for a one in 10 chance of cancer—would not benefit from the additional kallikrein measures.

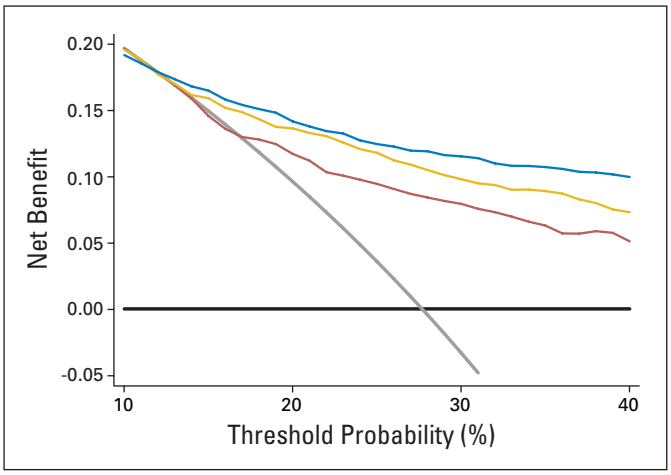

Fig 2. Decision curve analysis for the clinical model. The red line is for base model (age, digital rectal examination [DRE] result, and total prostate-specific antigen [PSA]); the gold line is for base model plus free PSA; and the blue line is for full model (age, DRE result, total PSA, free PSA, intact PSA, and kallikrein-related peptidase 2). As comparison, the gray line represents the strategy of biopsying all men, and the black line represents the strategy of biopsying no men. The line with the highest net benefit at a particular threshold probability will lead to the best clinical results.

For illustrative purposes, Table 3 lists the results of an approach where men would be biopsied if their predicted probability of prostate cancer was 20% or higher. We chose 20% as the probability threshold because this was close to the risk of cancer using the base model at the mean age of the sample and the widely used PSA cutoff of 4 ng/mL (21%). Therefore, Table 3 shows the results if the kallikrein panel was dichotomized into a simple normal versus abnormal result. If we were to biopsy only men with a predicted probability $\geq$ 20% from the full clinical model, for each 1,000 men with elevated PSA, we would conduct 513 fewer biopsies but advise against biopsy in 66 men with cancer. Of these 66 cancers, 54 would be Gleason grade $\leq$ 6, 10 would be Gleason grade 7, and two would be Gleason grade $\geq$ 8; in addition, 48 would be cT1c, 12 would be cT2a, five would be cT2b/c, and one would be cT3. Hence, the men recommended against immediate biopsy would have predominately low-stage and low-grade cancers.

Participants in this study received sextant biopsy. It is plausible that some participants with negative biopsy would have had cancer detected if an extended biopsy scheme had been used. To examine any possible effect of biopsy scheme on our findings, we examined participants who had a negative biopsy in the first round to determine the results of any biopsies during round 2, which occurred 4 years later. We then repeated our analyses assuming that those with a positive biopsy in the second round would have had cancer detected in the first round had they received an extended core biopsy. The same separation between training and validation sets was used. Of 526 men in the training set and 1,581 men in the validation set with initially negative biopsy, 204 and 633 men, respectively, were biopsied in round 2, of whom 20 and 75, respectively, were diagnosed with cancer. Although the estimates of predictive accuracy were slightly lower (laboratory models: 0.615 for base model *v* 0.757 for full model; clinical models: 0.672 for base model *v* 0.764 for full model; $P < .001$ for both), the increment in predictive accuracy associated with the full kallikrein model was similar (0.142 with round 2 biopsy data *v* 0.127 without repeat biopsy data for laboratory models; 0.092 with round 2 biopsy data *v* 0.081 without repeat biopsy data for clinical models). This suggests that our results are robust regarding the biopsy scheme.

Similarly, it might be suggested that our model would not be applicable for men with total PSA greater than 10 ng/mL because such men would be considered at high risk and be biopsied without need for additional markers. As such, we repeated all analyses excluding men with total PSA greater than 10 ng/mL. Restricting the range of total PSA reduced the predictive accuracy of all models, but the additional value of the panel compared with the base model was maintained; AUC increased from 0.566 to 0.725 and from 0.638 to 0.740 for the laboratory and clinical models, respectively.

DISCUSSION

We have replicated our previous finding that a panel of kallikrein markers can predict the result of prostate biopsy in previously unscreened men with elevated PSA. We also replicated our finding that application of a statistical model incorporating all four kallikreins would lead to superior clinical results compared with the current strategy of biopsying all men with elevated PSA; a large number of

Table 3. Biopsies Conducted and Cancers Found for Various Biopsy Strategies Applied to the Validation Set

Strategy	Biopsies			Cancers			High-Grade Cancers		
	Performed (No.)	Not Performed		Caught (No.)	Missed		Caught (No.)	Missed	
		No.	%		No.	%		No.	%
All biopsies	1,000	NA		277	NA		100	NA	
Laboratory models									
Total PSA ≥ 4 ng/mL	731	269	27	223	54	19	93	7	7
Risk ≥ 20%: total PSA, free PSA, age	618	382	38	222	55	20	92	8	8
Risk ≥ 20%: kallikrein panel, age	513	487	49	210	67	24	86	14	14
Clinical models									
Total PSA ≥ 4 ng/mL or positive DRE	794	206	21	244	33	12	95	5	5
Risk ≥ 20%: total PSA, free PSA, age, DRE	528	472	47	215	62	22	92	8	8
Risk ≥ 20%: kallikrein panel, age, DRE	487	513	51	211	66	24	88	12	12

NOTE. The risk models assume that men with a 20% or greater risk on the model would be biopsied. Numbers are given per 1,000 men with elevated PSA.
Abbreviations: NA, not applicable; PSA, prostate-specific antigen; DRE, digital rectal examination.

unnecessary biopsies could be avoided at the expense of only a small number of men with cancer being advised against biopsy, few of whom would have high-stage or high-grade disease. Accordingly, application of our model as part of PSA screening would reduce the harms associated with unnecessary biopsy.

The biology of prostate cancer and of the kallikreins is sufficiently well understood to provide plausibility to our panel. Transcription of PSA and hK2 is governed by androgens and that production is restricted to normal or malignant prostate epithelium.[11] Approximately one third of PSA released in seminal fluid comprises Lys_{145}-Lys_{146}–cleaved PSA, which is detected by free PSA but not by intact PSA assay, but no proPSA, which is detected by both intact and free PSA assays.[12] Findings that are apparently similar to ours have also been reported by others using, for example, a [–2]proPSA assay.[13]

Our study has several strengths. First, we have replicated a previously published study with similar results. In our previously published study with ERSPC Göteborg, the AUCs of the models using free and total PSA data obtained when the frozen samples were thawed to measure intact PSA and hK2 were similar (laboratory model: base = 0.658 and full = 0.774, increment of 0.116; clinical model: base = 0.703 and full = 0.786, increment of 0.083). Therefore, the current study is an independent replication of our prior findings. Second, we tested our statistical model with strict separation between the training and validation data sets. Third, we have shown, using decision analytic methods, that application of our markers would improve clinical decision making. Using our panel of four kallikreins would dramatically reduce the number of unnecessary biopsies, with only a small number of men with prostate cancer advised against immediate biopsy. Most of these men would have both low-stage and low-grade cancer. We anticipate that the men with cancers of higher stage or grade (including undergraded tumors) would have subsequent PSA increases, leading to biopsy and detection of cancer most likely at a curable stage. Fourth, our study cohort was a large, representative, population-based sample of the type of men to whom the marker panel would be applied in clinical practice.

However, perhaps the main strength of our study is its ready clinical applicability. Instead of tests that require novel clinical procedures (such as collection of urine after prostatic massage[14]) or laboratory tests (such as mass spectroscopy[15]), our panel could be implemented with no change to clinical practice (the same blood sample would be sent to a laboratory as for the current PSA test) and only a minor change to laboratory methods (it is relatively straightforward to convert the current PSA assay to a multiplex kallikrein assessment). The assays for the kallikreins have been refined over several years[8] and would be ready to implement clinically without significant or time-consuming further development. Moreover, the kallikrein panel seems to be highly cost effective; we have previously estimated that the panel of markers would add less than $100 to the cost of testing total PSA alone[6]; in comparison, the cost of biopsy is in the range of $1,000 to $2,000.

There are two major limitations of our study. First, fresh samples were not available for testing; thus, marker evaluation was conducted on frozen and rethawed samples. We have previously shown that repeated freezing and rethawing degrades kallikreins, affecting the predictive accuracy of the four-kallikrein panel. In particular, the use of frozen samples may explain why, in contrast to our prior report, hK2 and intact PSA did not enhance prediction of high-grade cancer. Second, our model was developed on men who had not been subjected to recent PSA testing. Nonetheless, using data from the second and later rounds of the Göteborg study, we have shown that the panel is highly predictive of biopsy outcome in recently screened men.[16] A similar consideration applies to repeat biopsy; our intention is to conduct further tests of the panel specifically in this population.

There are two possible criticisms of our study. The first might be that, given the lack of benefit found in the US Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial[17] and the large number of men needing to be screened and treated to prevent one death in the ERSPC,[2] PSA screening must be seen as unproven, suggesting that refinements to the PSA test are of merely academic interest. In response, we would argue that PSA testing is already widespread in the community; approximately 75% of US men have had a PSA test. This is unlikely to change dramatically in the light of the recent randomized data. As such, our approach is readily applicable to medicine as currently practiced. A second possible criticism of our study is that the key problem with PSA screening is overdiagnosis, rather than unnecessary biopsy. However, our approach would also lead to fewer cancers being diagnosed, with the vast majority of cancers not detected being low

stage and low grade, which are exactly the type of cancers thought to constitute overdiagnosis.

In summary, we have replicated our previous finding that adding information on kallikreins other than PSA can help predict the result of biopsy in men with elevated PSA. Thus, our models can be used to determine which men should be advised to have biopsy and which might be advised to continue screening but defer biopsy until stronger evidence of malignancy exists. Use of the models would reduce an important harm associated with PSA testing—the large number of unnecessary biopsies—and thus importantly shift the ratio between benefits and harms for this screening approach.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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